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Monte Carlo analysis of the human chlorpyrifos-oxonase (PON1) polymorphism using a physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model

C. Timchalk *, A. Kousba, T.S. Poet

Battelle Pacific Northwest Division, Chemical Dosimetry, 902 Battelle Blvd., P.O. Box 999, Richland, WA 99352, USA

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Abstract

Susceptibility to organophosphate (OP) insecticides, like chlorpyrifos (CPF), may result from differences in the extent of metabolic detoxification of the active metabolite, CPF-oxon. A genetic polymorphism in the arylesterase (PON1; CPF-oxonase) detoxification of OPs, results in the expression of a range of enzyme activities within humans. This study utilized Monte Carlo analysis and physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) modeling to investigate the impact of human CPF-oxonase status on the theoretical concentration of CPF-oxon in the brain. At low doses (~ 5 μg/kg) the model is insensitive to changes in CPF-oxonase. However, with increasing dose (> 0.5 mg/kg) the model suggests a dose-dependent non-linear increase in the brain CPF-oxon concentration, which is associated with CPF-oxonase activity. Following repeated high dose exposure, the model predicted brain CPF-oxon concentration was $\sim 8\times$ higher (5 mg/kg) versus a single exposure, whereas, at low doses (5 μg/kg), the brain concentrations were comparable regardless of exposure duration. This suggests that at low environmentally relevant exposures other esterase detoxification pathways may compensate for lower CPF-oxonase activity.

Keywords: Physiologically based pharmacokinetics; Organophosphate insecticide; Chlorpyrifos; PON1; Polymorphism

1. Introduction

Chlorpyrifos (*O*,*O*-diethyl-*O*-[3,5,6-trichloro-2-pyridyl]phosphorothioate) (CAS Registry No. 2921-88-2) is a thionophosphorus organophosphate (OP) and is the active ingredient in DURSBAN® and LORSBAN® insecticides. This broad-spectrum OP insecticide has seen widespread commercial application, and its primary toxicological effect is associated with the inhibition of acetylcholinesterase (AChE, EC 3.1.1.7) in both central and peripheral nerve tissues (Murphy, 1986; Sultatos, 1994).

The metabolic scheme for CPF metabolism is presented in Fig. 1. Phosphorothionates like chlorpyrifos (CPF) are only very weak inhibitors of AChE, but must first be metabolized to the corresponding oxygen analog (CPF-oxon), which

* Corresponding author. Tel.: +1-509-376-0434; fax: +1-509-376-9064

E-mail address: charles.timchalk@pnl.gov (C. Timchalk).

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has high inhibitory activity (Timchalk, 2001; Timchalk et al., 2002). The activation of CPF to CPF-oxon is mediated by cytochrome P450 mixed-function oxidases (CYP450) (Chambers and Chambers, 1989). In addition, oxidative dearylation of CPF to 3,5,6-trichloro-2-pyridinol (TCP) and diethylthiophosphate represents a competing detoxification pathway that is likewise mediated by CYP450 (Ma and Chambers, 1994). Hepatic and extra-hepatic A-esterase (PON1; CPF-oxonase) can effectively metabolize CPF-oxon without inactivating the enzyme (Sultatos and Murphy, 1983). In addition, B-esterases (B-EST) such as carboxylesterase (CaE, EC 3.1.1.1), and butyrylcholinesterase (BuChE, EC 3.1.1.8) can likewise detoxify CPF-oxon; however they become irreversibly bound (1:1 ratio) to the CPF-oxon and thereby become inactivated (Chandra et al., 1997; Clement, 1984). Susceptibility to OPs is primarily associated with differences in pharmacokinetics (i.e. absorption/distribution), metabolism (i.e. rate of oxon formation and degradation), and pharmacodynamic response (i.e. esterase inhibition) (Barron et al., 1991).

A physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model has recently been developed for CPF, CPF-oxon and TCP in rats and humans (Timchalk et al., 2002). In this model, the metabolism of CPF is described using Michaelis–Menten kinetics for CYP450 activation/detoxification as well as the CPF-oxonase mediated detoxification of CPF to TCP. Although, the PBPK/PD model has been validated against both rodent and human dosimetry and dynamic response data sets the impact of inter-individual variability in the model inputs (i.e. metabolism rates) was not fully evaluated.

A number of human and experimental animal studies have demonstrated a wide degree of variability in the metabolism of drugs and xenobiotics (Mackenzie et al., 2000; Tucker, 2000; Miller et al., 1997; Jones et al., 1995; Gonzalez and Gelboin, 1993). In this regard, a human genetic polymorphism in the PON1 detoxification of several OP insecticides, including the active metabolite of CPF, CPF-oxon has been well established, resulting in the expression of a range of PON1 enzyme activity within a segment of the population (Cowan et al., 2001; Furlong et al., 1988; Ecker-

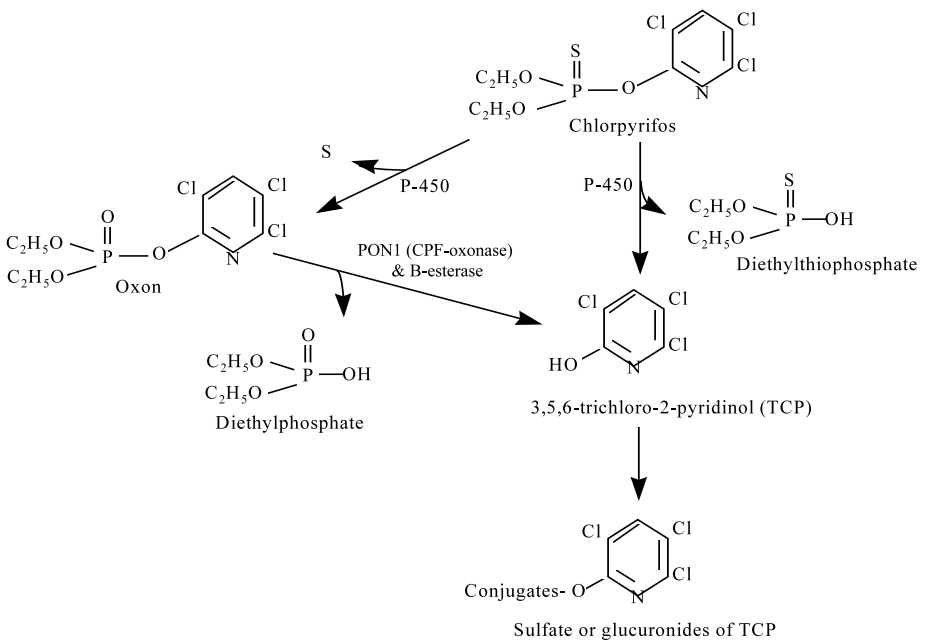

Fig. 1. Metabolic scheme for CPF, and the major metabolites CPF-oxon (oxon), TCP, diethylphosphate and diethylthiophosphate.

son et al., 1983; Geldmacher-von Mallinckrodt et al., 1983). The molecular basis of the polymorphism results from a single amino acid substitution, resulting in three PON1 genotypes, the RR and QQ homozygote, and the QR heterozygote (Furlong et al., 2000a). The rate of PON1 hydrolysis for the various polymorphic forms is substrate specific, with the R isoenzyme reported to have ~30% more activity towards CPF-oxon than the Q isoform (Davies et al., 1996). Richter and Furlong (1999) have noted the importance of considering both genotype and phenotype in assessing variability in PON1 status. Two-dimensional enzyme analysis was used to establish both PON1 phenotype and an accurate inference of genotype, for CPF-oxonase activity in a population of farm workers (Davies et al., 1996). The capability of PON1 to protect against OP toxicity has been demonstrated in several studies in which exogenous administration of PON1 provided protection against acute OP poisoning in rodents (Main, 1956; Costa et al., 1990; Li et al., 1993, 1995). In addition, recent studies in PON1-deficient mice indicate that they were more sensitive to the effects of OPs, including CPF and CPF-oxon than wild-type mice (Li et al., 2000; Furlong et al., 2000a; Shih et al., 1998). Cowan et al. (2001), recently utilized a gene delivery vector to incorporate the human PON1 genes into mice, boosting serum CPF-oxonase activity ~60%, which provided added protection against CPF-oxon AChE inhibition following in vivo exposure to a range of CPF doses. Since CPF-oxonase (PON1) is an important detoxification pathway for CPF-oxon, it is critical to understand the impact that inter-individual variability in human CPF-oxonase activity may or may not play in altering CPF-oxon dosimetry at environmentally relevant exposures.

A major objective for developing PBPK/PD models is to improve the accuracy of human health risk assessments for environmental contaminants (Clewell, 1995; Clewell and Andersen, 1996). To achieve this goal, it is critical to characterize the distribution of model responses that are associated with variability and uncertainty in key model parameters. In this regard, Monte Carlo methods have been applied to numerous PBPK models to help determine the overall impact of parameter variability and uncertainty on risk assessment predictions (Clewell and Andersen, 1996). Variability is a measure of inter-individual differences, whereas uncertainty relates to the potential error associated with parameter estimates (Clewell et al., 1999; Clewell and Andersen, 1996). It is important to recognize that there is no practical way to model variability and uncertainty separately from one another (Newtorov, 2001); however, by having well characterized parameter estimates it is possible to infer potential sources of variability or uncertainty.

The primary objective of this study was to evaluate the impact of variability associated with the CPF-oxonase polymorphism on the theoretical concentration of CPF-oxon in the human brain over a range of CPF doses. The approach entailed conducting a Monte Carlo analysis of CPF-oxonase using available measurements of enzyme activity reported for the QQ, QR and RR polymorphisms. This reported polymorphic distribution of CPF-oxonase activity was based on the in vitro metabolism of CPF-oxon in human serum obtained from a population of farm workers (Davies et al., 1996). It is anticipated that these model simulations can be used as the foundation for future experimental studies.

2. Materials and methods

2.1. PBPK/PD Model

The existing PBPK/PD model for CPF, as previously described (Timchalk et al., 2002), was used for the Monte Carlo analysis. Initial model simulations were restricted to single dose oral administration in humans, and the simulations were run through 24 h post-dosing. Once the most sensitive estimates of model parameters for CPF-oxonase activity were identified, a repeated dosing simulation was conducted using these parameters to further assess the impact of repeated exposure on the model response. The computer program SIMUSOLV®, which contains a numerical integration, optimization and graphical routine, and is based on the FORTRAN-based software ACSL

Table 1
Initial distribution of chlorpyrifos-oxonase (CPF-oxonase) enzyme activity (V_{maxC}) used for Monte Carlo analysis

Human CPF-oxonase polymorphism	Plasma V_{max} (μmol/l/min)[a]	Plasma V_{maxC} (μmol/h/kg)	Liver V_{maxC} (μmol/h/kg)[b]
QQ (low)	7484 ± 1840	68,821 ± 16,925	89,850 ± 22,095
QR (medium)	8152 ± 1519	74,983 ± 13,972	97,895 ± 18,241
RR (high)	9794 ± 2001	90,087 ± 18,406	117,614 ± 24,030

[a] Mean ± SD of human CPF-oxonase activities obtained from Davies et al. (1996).
[b] Human liver V_{maxC} was estimated based on the ratio of plasma to liver activity reported in rats (Mortensen et al., 1996).

was used for Monte Carlo simulations. The analyses were conducted at doses of 0.005, 0.05, 0.5 and 5 mg CPF/kg of body weight. These doses, range from above an effect dose for plasma ChE inhibition (>0.5 mg/kg) to below the reference dose (RfD) value (<10 μg/kg) for acute exposure (Nolan et al., 1984; Gibson et al., 1999).

2.2. Variability analysis (Monte Carlo simulations)

To assess the potential impact of the CPF-oxonase polymorphism on the theoretical concentration of CPF-oxon in the brain, a distribution of CPF-oxonase metabolic parameters for both the serum and liver enzymes for each genotype were determined by Monte Carlo analysis by adapting the PBPK/PD model as previously described (Thomas et al., 1996). In brief, the initial analysis step involved randomly sampling the CPF-oxonase substrate activities (V_{max}) to establish the enzyme distribution, which were then used as input parameters for the PBPK/PD model simulation. The enzyme activity distribution of CPF-oxonase in human serum were obtained from Davies et al. (1996), whereas, there were no available measurements of human liver CPF-oxonase activity. Therefore, it was assumed that the human liver CPF-oxonase would be $\sim 1.3\times$ greater than the serum activity, based on the ratio of these activities in the rat (Mortensen et al., 1996). Secondly, it was also assumed that the distribution of the polymorphic liver CPF-oxonase activities would be proportional to the serum activity, hence it was feasible to use the serum CPF-oxonase to estimate the liver enzyme distribution. These assumptions were reasonable since the CPF PBPK/PD model accurately simulated both rat and human pharmacokinetic and pharmacodynamic (i.e. esterase inhibition) response, and the human CPF-oxonase enzyme activities in the liver and serum were scaled from the rat parameter estimates (Timchalk et al., 2002). The probability distribution for the V_{max} values for CPF-oxonase were based on their means and standard deviations utilizing a normal distribution, and were consistent with the unimodal enzyme activity for CPF-oxonase initially reported by Furlong et al. (1988). The initial model parameters for CPF-oxonase activity used in the Monte Carlo simulations are presented in Table 1. At each dose level 1000 Monte Carlo simulations were run, utilizing the distribution of liver and serum CPF-oxonase activity for each genotype, the theoretical brain CPF-oxon area-under-concentration (AUC) curve was used as the dose metric for toxicity.

3. Results

A Monte Carlo analysis was conducted to assess the potential contribution of the human CPF-oxonase metabolic polymorphism to the inter-individual variability in susceptibility for toxicity to CPF. A comparison of the dose-dependent theoretical brain CPF-oxon AUC for the various CPF-oxonase polymorphisms are presented in Table 2. The result of these simulations suggests a dose-dependent non-linear increase in the theoretical brain CPF-oxon AUC, at doses >0.5 mg/kg, which appears to be a function of both dose and CPF-oxonase activity. At high doses (0.5–5 mg/kg), the analysis suggests greater variability (coefficient variation (cv) range from

32 to 56%) in the estimated dose metric (brain CPF-oxon AUC), including a larger spread between the minimum and maximum model response. In addition, at these higher doses, differences in the CPF-oxonase activity appear to have a substantially greater impact on brain dosimetry. For example, at 5 mg/kg there is nearly a doubling of the mean brain CPF-oxon AUC when comparing the QQ (low metabolizer) and RR (high metabolizer) polymorphism, and the maximum response was increased >3-fold. In contrast, at low environmentally relevant doses (5 μg/kg) there is considerably less variability in the estimated brain CPF-oxon AUC (cv range from 17 to 24%), and more importantly the model response is relatively insensitive to the variability in CPF-oxonase activity.

To further evaluate the potential implications of low CPF-oxonase metabolic activity, the impact of repeated dietary exposures over a range of CPF doses was considered. In these simulations the V_{maxC} parameters that resulted in the lowest CPF-oxonase activity were utilized in the simulation to represent a worst-case scenario, resulting in the highest theoretical brain concentrations of CPF-oxon (C_{max}). The comparison of the C_{max} simulations in humans following either a single or repeated (30 days) exposure is presented in Table 3. As expected, both single and repeated exposures result in a dose-dependent increase in the maximum CPF-oxon brain concentration. However, at low doses (5 μg/kg), repeated dietary exposure only minimally increase the C_{max} (ratio 1.2), whereas at higher doses (0.05–5 mg/kg), repeated exposures appreciably increase the C_{max} (ratios 1.6–8.4) relative to the single dose.

4. Discussion

The potential sensitivity of a given individual to the adverse health effects from exposures to envi-

Table 2
Comparison of theoretical brain-oxon AUC calculated utilizing Monte Carlo simulation for the QQ, QR, and RR PON1 polymorphism in humans following a single dose exposure to CPF at doses of 0.005, 0.05, 0.5 and 5.0 mg/kg

PON1	Theoretical brain CPF-oxon (AUC)[a,b]			
	CPF dose			
	0.005 mg/kg	0.05 mg/kg	0.5 mg/kg	5.0 mg/kg
QQ (*low*)				
Mean ± SD	0.06 ± 0.001 (1.2)	0.63 ± 0.16 (1.31)	11.6 ± 6.54 (1.63)	616 ± 333 (1.72)
cv	24%	26%	56%	54%
Maximum	0.15	1.66	72.9	4350
Minimum	0.03	0.36	4.64	245
QR (*med*)				
Mean ± SD	0.05 ± 0.01 (1.0)	0.57 ± 0.19 (1.19)	9.37 ± 3.16 (1.31)	492 ± 158 (1.38)
cv	17%	18%	34%	32%
Maximum	0.10	1.06	32.0	1600
Minimum	0.04	0.36	4.73	245
RR (*high*)				
Mean ± SD	0.05 ± 0.01	0.48 ± 0.10	7.11 ± 2.45	358 ± 123
cv	20%	20%	34%	34%
Maximum	0.09	0.99	26.7	1310
Minimum	0.03	0.29	3.57	171

Values in parenthesis represents the ratio of AUC for the QQ and QR relative to the RR polymorphism. cv, coefficient of variation.
[a] AUC = (μmol/l/h) × 10^{-6}.
[b] These theoretical AUCs are based upon model predictions, at these dose levels the CPF-oxon brain concentration is below the limits of quantitation (Timchalk et al., 2002).

Table 3
Comparison of the theoretical peak CPF-oxon brain concentration (μmol/l) in humans following a single or repeated (30 day) dietary exposure to chlorpyrifos (CPF) at doses of 0.005, 0.05, 0.5 and 5.0 mg/kg/day, utilizing the V_{maxC} PON1 (QQ polymorphism) parameter estimates that resulted in the highest brain CPF-oxon AUC

CPF (mg/kg)	Theoretical peak brain CPF-oxon (μmol/l)		
	Single	Repeat (30 days)	Ratio (repeat/single)
0.005	2.34×10^{-8}	2.71×10^{-8}	1.16
0.05	4.54×10^{-7}	1.03×10^{-6}	2.27
0.5	2.02×10^{-5}	3.16×10^{-5}	1.56
5.0	1.19×10^{-3}	9.96×10^{-3}	8.37

ronmental agents may be related to differences in metabolic capacity (Mackenzie et al., 2000; Miller et al., 1997; Jones et al., 1995). This is particularly true with OP insecticides where differences in the ratio of activation to detoxification are believed to be associated with chemical, species, and gender dependent sensitivity to OPs (Ma and Chambers, 1994). Although, both age-dependent and polymorphism specific increased sensitivity to OP insecticides have been demonstrated (Furlong et al., 1988; Mortensen et al., 1996; Pope and Liu, 1997; Atterberry et al., 1997), the importance of these differences for assessing real risk for individuals who are exposed to low environmentally relevant doses is unclear. In the current study, the dose- and time-dependent model sensitivity to changes in model parameters associated with inter-individual variability in human CPF-oxonase detoxification has been evaluated utilizing a PBPK/PD model to estimate the theoretically delivered dose of CPF-oxon to the brain.

The relevancies of the age and polymorphism dependent sensitivities to OP insecticides are questionable when considering real-world low-dose repeated exposures. In this regard, the Monte Carlo simulations for the CPF-oxonase polymorphisms (Tables 2 and 3) over a range of dose-levels, suggest that the response is relatively insensitive to changes in CPF-oxonase activity at low doses ($\sim$5 μg/kg), however, with increasing dose ($\sim$0.5–5 mg/kg) CPF-oxonase status may be an important determinant of sensitivity. These results suggest that other esterase detoxification pathways may adequately compensate for lower CPF-oxonase activity; hence an increased sensitivity to low CPF-oxonase is not observable until non-target esterases have been appreciably depleted (see Fig. 2). This response is also consistent with experimental results and the observed model simulations that show a non-linear response at CPF doses that significantly deplete ($>90\%$ inhibition) plasma BuChE activity in humans (Timchalk et al., 2002).

Furlong et al. (2000b), has utilized a knockout mouse to further evaluate the in vivo role of PON1 in providing protection against OP toxicity. Shih et al. (1998) has shown the PON1-knockout mouse, to be particularly sensitive to CPF-oxon ChE inhibition relative to the wild-type animals. This increased sensitivity appears to be associated with an $\sim30\times$ decrease in CPF-oxonase activity in the knockouts relative to wild-type mice (Li et al., 2000; Furlong et al., 2000a). Since it is not ethically feasible to assess the human PON1 polymorphism following high dose in vivo exposures, the knockout mouse represents an ideal in vivo model system where it is feasible to replace the mouse PON1 with the different human PON1 isoforms. Injection of purified human PON1 isoforms in the PON1-knockout mice (Li et al., 2000) or recombinant injection of human PON1 isoform genes into mice demonstrated an increased CPF-oxonase activity. In addition, as observed in vitro with human serum (Davies et al., 1996) injecting knock-out mice with the purified human PON1 isoforms resulted in the

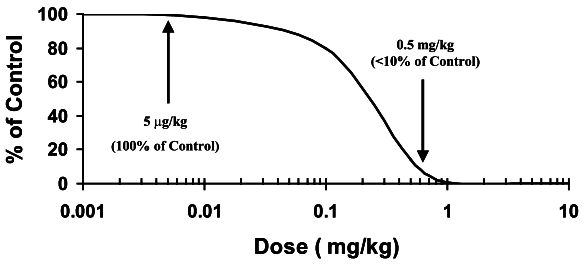

Fig. 2. Simulation of peak plasma BuChE inhibition dose-response in humans following an acute exposure to a broad range of CPF doses.

RR isoform hydrolyzing CPF-oxon at a slightly faster rate (1.7 ×) than the QQ; which was consistent with the observed in vitro human results used for the current Monte Carlo analysis. Most recently, Furlong et al. (2002) has developed a transgenic mouse model that expresses the human PON1 polymorphism. Initial studies indicate that these mice equally express either isoform (RR and QQ), and mice expressing the RR isoform demonstrated greater resistance to CPF-oxon toxicity following dermal exposure. These results are consistent with the higher efficiency of CPF-oxon hydrolysis by humans with the RR PON1 polymorphism (Davies et al., 1996; Li et al., 2000). The development of the PON1 knockout and transgenic mouse models makes it feasible to conduct a number of in vivo experiments, which can be utilized to further validate the predictive capability of the PBPK/PD over a range of relevant human CPF-oxonase activities.

Although, the current model simulations illustrate the potential contribution of the PON1 polymorphism to inter-individual variability, it needs to be remembered that this is but one of several enzyme systems involved in the metabolism of CPF. Individual sensitivity to a given OP insecticide will depend upon the balance between delivered dose and the overall rates of metabolic activation and detoxification (Timchalk, 2001). Hence, the coupling of Monte Carlo analysis, with PBPK/PD modeling provides a unique tool for assessing the quantitative impact of parameter variability on individual risk (Clewell and Andersen, 1996).

In summary, this study was conducted to investigate the impact of CPF-oxonase status on the theoretical concentration of CPF-oxon in the human brain over a range of CPF doses. The impact of the human CPF-oxonase metabolic polymorphism on CPF metabolism and detoxification was evaluated using Monte Carlo analysis and suggests that the polymorphism has the greatest impact on target tissue dosimetry at dose levels which overwhelm other detoxification pathways (i.e. non-target esterases). Finally, this study also illustrates the potential utility of PBPK/PD modeling as a tool for determining the overall impact of parameter variability on risk assessment predictions for OP insecticides.

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